contributes to GABAR subunit-subunit interfaces. We studied gating properties and surface expression of wild type (wt) α6β2γ2 and mutant α6(Q237R)β2γ2 receptors expressed in HEK293T cells. Transient currents were evoked using ultrafast application of 400-μsec pulses of saturating GABA (1 mM) applied to excised outside-out macropatches. Peak amplitudes of α6(Q237R)β2γ2 currents were not reduced but α6(Q237R)β2γ2 currents had slower activation (~1.9 msec), slower and more desensitization (~60 %), and faster deactivation (~2-fold) than wt currents. On-cell α6(Q237R)β2γ2 and α6β2γ2 single channel currents had similar mean open durations, but opening and burst frequencies were significantly higher for mutant than for wt currents. We assessed surface and total cellular expression of wt and mutant receptors using flow cytometry. Co-expression of α6(Q237R) with β2 and γ2L subunits resulted in no significant reduction in total or surface expression of any subunit. We concluded that this mutation alters gating, but not trafficking, of α6β2γ2 GABARs.

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A GABA-A ligand Gated ion Channel Screen - results and Best Practices

Cristian Ionescu-Zanetti, Qin Chen.

The $GABA_A$ receptors belong to a family of ligand-gated ion channels mediating fast synaptic transmission. They are drawing great attention in pharmaceutical field to their potential roles in the development of new therapeutics affecting anxiety, sleep disorders, and muscle relaxation. However, ligand gated ion channel screening has been hampered by the lack of suitable high throughput electrophysiology platforms. While some studies have shown that it is possible to record the GABA tail current and use that information during a screen, such methods have and inherently lower signal to noise ratio and cannot be used on faster desensitizers.

Here we present a the use of a novel electrophysiology screening platform integrating a microfluidics network for the study of $GABA_A$receptor pharmacology. This platform features fast (<100ms) solution exchange coupled with simultaneous data recording. A novel assay could monitor GABA response in real time, and obtain a 3 point EC_{50} dose curve within 1 minute.

The $GABA_A$ α1β3γ2 expressing HEK cells from Millipore were used for this study. The channel was targeted with agonists, including GABA and muscimol, inhibitors (picrotoxin, bicuculline, and gabazine), and positive modulators, including diazepam, zolpidem and chlordiazepoxide. The positive modulators produced concentration dependent augmentation of the GABA EC_{20} response. The pharmacology data determined using this method was consistent with the literature values obtained using other platforms. Statistical data for inter and intra-plate reproducibility, current stability, and Z-values, is used to validate this approach.

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Assigning Functional States to Structural Models of Nicotinic-Receptor Type ion Channels

Giovanni Gonzalez-Gutierrez, Claudio Grosman.

Aromatic-aromatic interactions are a prominent feature of the crystal structure of ELIC a bacterial member of the nicotinic-receptor superfamily of ion channels where five pore-facing phenylalanines come together to form a structure akin to a narrow iris that occludes the transmembrane pore. To identify the functional state of the channel that this structure represents, we engineered phenylalanines at various pore-facing positions of the muscle acetylcholine receptor, including the position that aligns with the native phenylalanine 246 of ELIC, and assessed the consequences of such mutations using electrophysiological and toxin-binding assays. From our experiments, we conclude that the interaction among the side chains of pore-facing phenylalanines leads to the formation of a non-conductive conformation that is unresponsive to the application of acetylcholine and is highly stable even in the absence of ligand. Moreover, electrophysiological recordings from a GLIC channel (another bacterial member of the superfamily) engineered to have a ring of phenylalanines at the corresponding pore-facing position suggest that this novel refractory state is distinct from the well-known desensitized state. It seems reasonable to propose, then, that it is in this peculiar non-conductive conformation that the ELIC channel was crystallized. It seems also reasonable to propose that, in the absence of rings of pore-facing aromatic side chains, such stable conformation may never be attained by the acetylcholine receptor. Incidentally, we also noticed that the response of the proton-gated, wild-type GLIC channel to a fast change in pH from 7.4 to 4.5 (on the extracellular side) is only transient, with the evoked current fading completely in a matter of seconds. This raises the possibility that the crystal structures of GLIC obtained at pH 4.0 and pH 4.6 correspond to the (well-known) desensitized state.

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Conformational Dynamics in a Nicotinic Receptor Homologue Probed by Simulations

Hugues Nury, Frédéric Poitevin, Catherine Van Renterghem, Toby Allen, Jean-Pierre Changeux, Pierre-Jean Corringer, Marc Delarue, **Marc Baaden**.

Recently discovered bacterial homologues of eukaryotic pentameric ligand-gated ion channels (LGICs) are increasingly used as structural and functional models of signal transduction in the nervous system. The available structural knowledge of LGICs increased lately with two crystal structures of bacterial homologs in distinct conformations. We crystallized the receptor from the bacteria Gloeobacter violaceus (GLIC), which is gated by protons, at acidic pH [1]. The structure reveals an open pore and molecular dynamics simulations suggest that the protein is stable on a 20 ns timescale when embedded in a lipid bilayer [1]. It can undergo large motion at neutral pH, and we will present a one-microsecond long molecular dynamics simulation of the GLIC channel pH stimulated gating mechanism [2]. The crystal structure of GLIC obtained at acidic pH in an open channel form is equilibrated in a membrane environment and then instantly set to neutral pH. The simulation shows a channel closure that rapidly takes place at the level of the hydrophobic furrow and a progressively increasing quaternary twist. The observed transitions suggest a possible two-step domino-like tertiary mechanism that takes place between adjacent subunits.

Further simulations are underway to better understand the influence of side-chain protonation states on the open state of this receptor at acidic pH, as well as its interactions with general anesthetics.

[1] N. Bocquet et al., Nature 457, 111-114 (2009)
[2] H. Nury et al., PNAS 107, 6275-80 (2010)

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Structural Rearrangements Underlying Mg2+ Dependent Gating in Cora

Olivier Dalmas, Eduardo Perozo.

The recent structures of CorA from Thermotoga maritima have provided an excellent model for a molecular understanding of Mg2+ transport. So far all crystal structures, obtained at high divalent cation concentrations, appear to be in a ''closed'' conformation. Macroscopic currents recorded from oocytes macropatches show that Mg2+-binding to the cytoplasmic side may act as a gating factor, defining CorA as a Mg2+-activated, Mg2+-channel. The role of the putative Mg2+ sensor was tested by engineering a salt bridge between residue D253 and D89 at the interface of the each monomer. The presence of a positive charge at either residue was sufficient to lock CorA in a closed conformation independent of the free [Mg2+].

Using site-directed spin-labeling EPR spectroscopy, we were able to detect Mg2+-dependent conformational transitions with an apparent KD close to 2 mM, a concentration in the physiological range of free [Mg2+] in cells. The structural rearrangements associated with CorA gating were measured on 106 positions along the stalk helix and TM1 segment, near the fivefold axis of symmetry. Strikingly, a significant increase in the spin-spin dipolar coupling at the tip of the stalk helix was detected from CW-EPR and DEER measurements. Mapping these EPR-determined conformational changes on the CorA crystal structure suggest an explicit structural mechanism for CorA opening, where an umbrella-like closing motion of the stalk helix would lead to an expansion of the pore immediately after the kink. This conformational wave propagate to the pore forming helix TM1 that rotates, ultimately increasing the diameter of the permeation pathway. This mechanism is supported by an increase in both, NieddA accessibility and probe mobility with a concomitant decrease in spin-spin coupling along the permeation pathway. Altogether, these results suggest a plausible molecular mechanism of gating in Magnesium channel related to CorA.

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Estimation of ion Channel Kinetics from Macroscopic Recordings

Luciano Moffatt.

A sizeable portion of the kinetic information present on macroscopic recordings is discarded by standard statistical analyses based on least-squares minimization. A more general approach that uses all the kinetic information present in the recording consists in maximizing the likelihood function, the probability of obtaining the data as a function of the parameters of the kinetic model.

The exact likelihood function can be calculated only for a very small number of channels. Approximations proposed for preparations above 30 channels work fine when the acquisition time is smaller than the time the preparation needs to change its state, but this is not usually the case on experimental recordings. To overcome this limitation we developed the Integrated Macroscopic Recursive algorithm an approximation that can be applied to experimental data.

This algorithm assumes for each measurement an a priori knowledge of the possible state of the ensemble of channels at the beginning and at the end of